

EXPERIMENTAL STUDIES ON THE LIFE
HISTORY OF A ROTIFER REPRODUCING
PARTHENOGENETICALLY (PROALES
DECIPIENS)

BY
BESSIE NOYES

A DISSERTATION

Submitted to the Board of University Studies of the Johns Hopkins University
in conformity with the Requirements for the degree of
Doctor of Philosophy

BALTIMORE, MD.

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EXPERIMENTAL STUDIES ON THE LIFE-HISTORY OF A ROTIFER REPRODUCING PARTHENO- GENETICALLY (*PROALES DECIPIENS*)

BESSIE NOYES

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INTRODUCTION

Proales decipiens, a small rotifer belonging to the family of Notommatidae, presents unusual opportunities for studies on the life-history of the Rotifera, for work on long-continued parthenogenesis, and particularly for attempts to alter racial characteristics by selection or by environmental action. The animal lives in cultures of decaying vegetable material; it thrives in the hay infusions, malted-milk solutions, and the like, commonly employed for cultures of *Paramecium*. It is extremely hardy, readily lends itself to culture of isolated individuals on hollow-ground slides, and thrives under wide ranges of environment. At laboratory temperature it begins to produce eggs twenty-four to thirty-six hours after hatching. The number is low on the first day, increases gradually until the fifth day, then declines sharply, and egg production ceases on the seventh or eighth day, and the death of the adult follows in one or two days. In a typical life-cycle an egg deposited by the mother hatches in twelve to twenty-four hours; the embryo reaches the egg-laying period in twenty-four to thirty-six hours after hatching and

Five months later (in June, 1919), among another 100 organisms isolated under the same experimental conditions, 42 lived for 7 days, 39 for 6 days, 15 for 5 days, 2 for 4 days, and 2 for 3 days, giving an average length of life of 6.17 days. This increase in the length of life, accompanied by a marked increase in the number of eggs deposited, formed the basis for an experiment in selection in an attempt to increase both of these factors beyond the limits indicated above, as detailed in a later section. A tabulation of 1200 individuals reared at laboratory temperature (47° to 63°F.) in $\frac{1}{16}$ per cent malted milk at various times during the study shows that 295 lived for 7 days, 357 for 6 days, 382 for 5 days, 127 for 4 days, 35 for 3 days, and 4 for 2 days, giving an average length of life of 5.60 days (table 1). Under these conditions, with food constant and temperature variable, the individual range of life was from two to seven days with an average life cycle of 5.47 days for 1400 individuals. In an experiment with 254 organisms isolated in $\frac{1}{16}$ per cent malted milk and kept constantly at 23° to 25°C., it was found that 9 lived for 7 days, 56 for 4 days, 84 for 5 days, 60 for 4 days, 33 for 3 days, 10 for 2 days, and 2 for 1 day, giving an average length of life of 4.68 days.

TABLE 1

A table of the length of life of 1200 individuals (100 in a group) reared in $\frac{1}{16}$ per cent malted milk at laboratory temperature

2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	TOTAL
	4	27	69			100
	2	2	15	39	42	100
	8	26	66			100
	2	2	15	39	42	100
2	5	19	34	29	11	100
	2	4	27	38	29	100
	2	8	20	29	41	100
	3	9	23	34	31	100
	2	5	29	33	31	100
1	2	10	24	34	29	100
1	3	7	32	42	15	100
		8	28	40	24	100
Total, 4	35	127	382	357	295	1200

Thus, under conditions of constant temperature and food, the average length of life is lower than the average for individuals kept under conditions of constant food supply and fluctuating temperature.

As all these records were from isolated individuals, in many of the cases the short life of an individual may have been due to a slight mechanical injury inflicted when the organism was transferred from one depression slide to another in the daily routine; this is particularly likely to occur towards the close of the life-cycle when the posterior part of the body has become inflated and lost the power of movement.

When the egg is deposited in the water there is no indication of movement in the embryo; but six to eight hours before hatching the embryo is clearly distinguishable and can be observed as it turns within the egg; the jaws are in constant motion, striking out against the membrane apparently in an attempt to force a place of exit. Just before the individual emerges the egg membrane bulges outward near the larger end of the egg and immediately over the jaws of the embryo; this bulge increases until the membrane ruptures, leaving an irregular opening through which the organism escapes. The first definite bulging of the egg membrane occurs about two minutes before the organism escapes into the water. After emerging, the young individual moves rapidly, at random, through the water, twisting and bending sharply in its course. The body increases gradually in length and diameter for the first four days. At this time the maximum length is reached in most cases, but the increase in diameter continues until a day or two before death.

At room temperature the organism begins to deposit eggs about twenty-four hours after it has hatched, and one to two days before the maximum length of the body is attained. At this time a yellow-brown substance begins to appear in the posterior three-quarters of the body; this substance increases in amount until the body becomes brownish in color, the posterior part becomes inflated, and most of the power of movement is lost, owing to the increase in size and the loss of flexibility. In isolated individuals random movement ceases about the second

day of the egg-laying period and the adult takes a circular path near the edge of the medium; as a result of this course, the eggs are deposited near the edge of the liquid and usually occur in two or three groups. The act of depositing eggs has never been observed, so that it is not known whether these groups represent two or three separate deposits or whether the eggs are deposited singly and the groupings are accidental and the result of the circular path taken up by the mother. In the young, transparent organism the passage of food through the jaws, through the esophagus and back into the body, and finally the excretion of waste products can be traced with ease. About the time of death, however, excretion ceases, movement is confined to the anterior cilia and jaws, and the action of the jaws in grasping food seems to be ineffective. Throughout the entire life-cycle the extrusion of waste products from the body seems much less than the amount of material taken through the jaws would indicate, and during the last two days of the life-period many specimens cease the extrusion of fecal masses entirely. At the close of the life-cycle the organism differs markedly from the adult early in the egg-laying period.

The organism furnishes excellent material for a study of the physiological and structural changes accompanying maturity, old age, and death. The transparency of the embryo with its rapid development and the accompanying change in color offers excellent opportunity for a study of the relations of the retention of the products of metabolism, muscular degeneration, and the effects of aging.

The usual type of egg deposited by *Proales* is slightly larger at one end, whitish in color, very thin-shelled, and $48 \times 80\mu$ in size. The egg increases very little in size before hatching, but a few hours after it has been deposited the outline of the embryo can be distinguished. This outline increases in distinctness, and soon the embryo can be seen flexed on itself with the long axis parallel to the long axis of the egg. In over 50,000 eggs observed in fresh culture medium, hatching has taken place in all cases within twenty-four hours, with the exception of five individual eggs which appeared to have been slightly injured

when the mother was removed from the slide and which disintegrated very soon. If eggs are left in isolation slides in old culture medium, hatching frequently does not take place at all; a cloudy, irregular, zone appears around the egg and disintegration soon follows.

In February, 1919, immediately after the study was begun, sixteen eggs quite different from the usual type described above were deposited. These eggs were $90 \times 150\mu$ in size, yellowish brown in color, with a lighter yellow circular area in the center, blending to a dark brown at either end. The shell or egg membrane was very thick. All of these eggs were kept in fresh culture medium for three months, under conditions in which the thin-shelled forms hatched readily. No change in appearance had taken place during this time, so they were separated into two lots; one lot was subjected to a temperature of $\pm 6^{\circ}\text{C}$. for a period of two days, then returned to the temperature of the laboratory; the other lot was kept at 36°C . for an equal length of time and returned to the laboratory temperature, but no change followed this period of increase and decrease in temperature. Finally, all the eggs were dried for two weeks at laboratory temperature and then returned to fresh culture solution, but as no change could be detected, they were discarded at the end of six months. No eggs of this type have appeared during the later study of the organism, lasting over a period of thirteen months.

In many species of rotifers two kinds of eggs are produced, thin-shelled ones during periods favorable for rapid multiplication, and thick-shelled forms at the onset of unfavorable conditions; these later are known as the 'winter' or 'resting' form. In *Hydatina senta*, studied so extensively by Whitney and by Shull, three forms are produced, a large thin-shelled form which develops at once into a female, a small thin-shelled which develops at once into a male, and a thick-shelled form which develops after a period of rest into a female; the first two forms are produced parthenogenetically, the third by a fertilized female. The thick-shelled eggs of *Proales* are comparable to the 'winter' eggs described above, since they appeared at a time when reproduction under normal conditions might be accomplished

with difficulty; but such a comparison must be made with reservation, owing to the absence of any such eggs during the winter of 1919-1920, although some cultures were under the same conditions of environment as during the previous winter. On the other hand, such thick-shelled forms may have been deposited by fertilized females and have never appeared in later cultures, since all forms produced in malted milk have been females and there has hence been no possibility of fertilization taking place.

An individual usually deposits an egg during the first twenty-four hours of its life, and the average daily rate of production determined for 1500 individuals isolated in $\frac{1}{16}$ per cent malted milk was 1 egg the first day, 3 the second, 5 the third, 7 the fourth, 3 the fifth, and 1 the sixth. There is, however, much variation in different individuals, as will be seen from table 2, which gives the daily egg production for fifty specimens in February, 1919, and for fifty specimens five months later in June, 1919.

As the table shows, there is an individual range of about 1 to 10 eggs for every day of the egg-laying period. If the number of eggs deposited on the first day is high (7 to 9), the life-cycle is usually shortened to three to five days, but if the number is small (1 to 3) on the first day and increases gradually on the second and third days, the life-cycle is longer (five to seven days) and in many cases the egg production higher. For 1200 individuals in $\frac{1}{16}$ per cent malted milk at room temperature (47° to 63°F.) the average total egg production was 19.56. For 254 individuals in $\frac{1}{16}$ per cent malted milk at a constant temperature of 23° to 25°C. the average total egg production was 17.37. This decrease in number of eggs was associated with a decrease in the average length of life from 5.60 days to 4.68 days, as has been noted earlier in the paper. For 100 individuals isolated early in the study the average total egg production was 12.79, while the average production of an equal number of individuals a few months later was 23.43 eggs per individual. This increase in average egg production was associated with an increase in the average length of life from 4.65 days to 6.17 days. This marked difference in egg production and length of life in two groups of

organisms at different times in the history of the race suggested the possibility of increasing these two factors still further by artificial selection; an experiment with this in view is described later.

The early production of eggs, the large number of eggs produced, the high percentage of viability in fresh malted-milk solution, together with the short length of the average life-cycle, give us a race of organisms increasing rapidly. In 100 trials made during the study, a single individual, isolated in a small watch-glass, supplied daily with fresh malted milk and allowed to reproduce for seven days, showed an average of 125.63 descendants (adults, young, and eggs), including members of four generations. During the thirteen months the race has been under observation over 125,000 individuals have been studied in isolation; all of these organisms have produced eggs of the thin-shelled variety, with the exception of the sixteen thick-shelled forms mentioned earlier. The fact that only females had been produced and the absence of the two types of eggs in the later work led to a search for the male form and the experimental conditions under which it appears, as set forth in the next section.

DO MALES OCCUR? AN EXPERIMENTAL STUDY

As has already been set forth in this paper, the line of descent in *Proales* consists mainly of females reproducing parthenogenetically. Is this the only method of reproduction? Do males ever appear, as in most other species of rotifers? In a number of species the male occurs either continuously or at irregular intervals in the life-history, but in one large group, the *Bdelloida*, no males are known and reproduction takes place continuously by parthenogenesis. In the genus *Proales* males have been observed for two of the four species; in *Proales werneckii*, which lives in galls of *Vaucheria*, the male resembles the female closely in size and form, although the alimentary canal is not so fully developed; in *Proales parasita*, Plate has recorded the appearance of a male of the usual reduced size and structure; in *Proales decipiens* and *Proales gigantea* the male has not been recorded.

TABLE 2

Daily egg production of 100 individuals of Proales decipiens, cultivated in $\frac{1}{16}$ per cent malted milk

A. FIFTY INDIVIDUALS IN FEBRUARY, 1919							B. FIFTY INDIVIDUALS IN JUNE, 1919										
INDIVID- UAL	Days						INDIVID- UAL	Days									
	1st	2d	3d	4th	5th	To- tal		1st	2d	3d	4th	5th	6th	7th	To- tal		
1	5	3	3	d		11	1	2	2	6	7	5	5	d	27		
2	4	2	2	2	d	10	2	1	5	7	5	4	2d		26		
3	3	1	1	2	4d	11	3	1	4	8	6	3	1	d	23		
4	1	1	2	3	d	7	4	1	7	6	6	4	2	d	26		
5	4	2	3	1	1d	11	5	2	6	5	7	3	1	1d	25		
6	5	1	2d			8	6	1	8	7	5	4	2d		27		
7	7	3	d			10	7	3	6	7	d				16		
8	8	4	0	2	1d	15	8	2	7	6	4	1	d		20		
9	9	6	0	4	2d	21	9	2	6	7	6	3	2d		26		
10	0	0	10	2	d	12	10	4	6	7	8	2	d		27		
11	2	2	3	3	11d	21	11	3	6	8	7	2	1	d	27		
12	12	2	2	2	1d	19	12	2	6	6	7	4	2	1d	28		
13	1	3	3	4	1d	12	13	1	5	7	5	2	1d		21		
14	2	1	2	2	d	7	14	2	9	5	7	3	0	d	26		
15	2	2	6	1	2d	13	15	2	8	4	3	3	3d		23		
16	1	1	3	3	2d	15	16	1	9	5	2	1d			18		
17	5	3	0	d		8	17	1	5	6	7	4	3	d	26		
18	2	3	0	3	1d	9	18	2	6	6	3	2	1d		20		
19	4	1	5	4	4d	20	19	1	7	8	9	1	d		26		
20	1	4	5	3	2d	15	20	0	8	8	7	4	2	1d	30		
21	1	2	5	4	1d	13	21	0	7	5	3	d			15		
22	5	4	3	4	2d	18	22	3	9	7	6	2	1d		28		
23	0	3	3	4	1d	11	23	2	6	7	8	3	2	1d	29		
24	3	4	2	4	1d	14	24	1	5	9	8	2	1d		26		
25	2	3	6	5	2d	18	25	3	6	5	4	6	0	3d	27		
26	0	2	4	6	3d	15	26	1	7	6	3	6	2d		25		
27	3	4	5	6d		18	27	2	5	7	6	4	1	d	26		
28	0	4	2	7	3d	16	28	5	6	4	3	1d			19		
29	2	3	7	1	2d	15	29	1	6	4	5	7	0	d	23		
30	1	3	5	4	d	13	30	4	7	5	4	5	0	d	25		
31	1	3	2d			6	31	3	8	2	6	5	1	1d	26		
32	1	2	6	8	3d	20	32	2	7	7	7	5	d		28		
33	1	4	5	6d		16	33	2	6	5	4	1d			18		
34	1	2	6	8	d	17	34	2	6	9	5	2	4d		28		
35	0	3	4	5	6d	18	35	2	6	5	5	4	0	d	22		
36	1	4	4	6d		15	36	1	7	8	6	5	0	d	27		
37	1	4	4	8	1d	18	37	3	4	9	5	5	01d		27		
38	3	2	5	6d		16	38	1	2	5	9	5	1	1d	24		

TABLE 2—*Concluded*

A. FIFTY INDIVIDUALS IN FEBRUARY, 1919							B. FIFTY INDIVIDUALS IN JUNE, 1919								
INDIVID- UAL	Days						INDIVID- UAL	Days							
	1st	2d	3d	4th	5th	To- tal		1st	2d	3d	4th	5th	6th	7th	To- tal
39	1	3	6	6	2d	18	39	2	7	6	5	4	d		24
40	1	2	0	7	3d	13	40	1	8	7	5	3d			24
41	1	3	3	7	1d	15	41	1	9	10d					20
42	1	2	1	2	4d	10	42	1	3	5	7	4	2	1d	23
43	1	4	4	6	d	15	43	1	7	6	8	2	0	d	24
44	1	1	1	1	d	4	44	2	5	6	6	4	1d		24
45	1	3	0	d		4	45	3	6	9	7	1d			26
46	1	4	5	6	7d	23	46	1	10	10	2	1	0	d	24
47	1	4	6	8d		19	47	3	7	10	3	2	1d		26
48	1	3	4	5	2d	15	48	2	9	9	5d				25
49	1	3	5	7d		16	49	5	6	9	5	1	0	d	26
50	0	2	1	2d		5	50	3	9	9	6	2	0	d	29

d indicates day of death.

In most of the species of rotifers, the males differ markedly from the females; they are smaller, shorter-lived, much more active than the younger females, and are usually degenerate in structure, lacking complete excretory or digestive systems. But in *Proales werneckii* the male is of the same size as the female and resembles her externally. On this account it seemed inadvisable to rely on appearances alone as to whether the form under observation in *Proales decipiens* was male or female, but if on isolation eggs were deposited it was clear that the individual was female. The appearance of two types of eggs, thin-shelled and thick-shelled, early in the history of the race suggested that at that time males might be present and the thick-shelled eggs deposited by a fertilized female. This suggestion was supported further by the fact that the thick-shelled eggs did not appear during the second winter, as they perhaps would had they been merely 'winter eggs.' Early in the experiment it was noticed that in the usual culture fluid of $\frac{1}{16}$ per cent malted milk, all individuals isolated for any experiment produced eggs; that is, in malted milk no males appeared. In the case of

Hydatina senta, Shull and Whitney have shown that various factors in the environment have an influence upon the proportion of males appearing in any line. The amount of oxygen in the medium, the concentration of the food, the proportion of various microorganisms available for food, and the concentration of various chemicals seem to be factors associated with the percentage of males, or of male-producing females, produced by various mothers.

In Proales, changes in the nature and concentration of the food, changes in the temperature at which various cultures were maintained accompanied by changes in food, and changes in the chemical constitution of the medium were introduced in the study of the conditions under which the male form might be produced. In a culture fluid of $\frac{1}{16}$ per cent malted milk, female forms had consistently appeared throughout the study; so increases in the concentration of the milk were made until solutions of $\frac{1}{8}$ per cent, $\frac{1}{4}$ per cent, $\frac{1}{2}$ per cent, and 1 per cent had been used. In the higher concentrations bacteria developed so rapidly that the medium soon became flocculent and less favorable for a high egg production and greater length of life. On the other hand, a decrease in the concentration of the milk to $\frac{1}{32}$ per cent and $\frac{1}{64}$ per cent tended to decrease the available food to such an extent that the number of eggs deposited decreased, although the length of the life-cycle was not influenced. Males do not appear in malted milk either in decreased or increased concentrations. Beef extract, made from Armour's bouillon cubes, was made in $\frac{1}{8}$ per cent, $\frac{1}{4}$ per cent, and $\frac{1}{2}$ per cent concentration. The number of eggs produced in any of these percentages was much lower than in the stock solution of malted milk and in no case was there any change in the nature of the eggs or the organisms produced. Pasteurized milk in $\frac{1}{16}$ per cent, and $\frac{1}{8}$ per cent concentrations was employed, but owing to rapid bacterial action the solution became flocculent within a very few hours at laboratory temperature; successive generations of rotifers were maintained in it with difficulty and no males were produced. A solution of horse manure made after Whitney's ('14) formula was prepared and used in the proportion of 1 part horse-manure

solution to 3, 4, 5 and 6 parts of spring water. Egg production and the average length of life were both much reduced in this medium and no change in the mature form or egg were produced. At various times throughout the experiment *Proales* was cultivated in spring water in which various unicellular green plants and animals were flourishing, but in all cases isolated adults produced eggs of the usual thin-shelled form. In all cases isolations were made for five successive generations, since Shull ('12) has shown that the action of the environment is not accompanied by a change in sex until grandchildren are produced. None of the changes in the nature or concentration of the food were accompanied by the appearance of the male form in *Proales*.

Cultures in $\frac{1}{16}$ per cent malted milk, $\frac{1}{4}$ per cent beef extract, and in $\frac{1}{16}$ per cent pasteurized milk were subjected to a constant temperature of $\pm 6^{\circ}\text{C}$., 17°C ., 24°C ., and to a fluctuating temperature of 13° to 23°C . without producing any change in the uniform female constitution of the population.

In the summer of 1919, a number of mass cultures were established in small watch-glasses; in each of these cultures there was 5 cc. of malted milk with sufficient of a $\frac{1}{10}$ N solution of the following chemicals to give the percentage (of $\frac{1}{10}$ N) indicated.

HCl, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, 2, 3 per cent	KCl, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, 2, 3 per cent
H ₂ SO ₄ , $\frac{1}{2}$, $\frac{3}{4}$, 1, 2, 3 per cent	MgSO ₄ , 2, 4, 6 per cent
NaCl, 2, 4, 6 per cent	K ₂ HPO ₄ , $\frac{1}{2}$, $\frac{3}{4}$, 1, 2, 3 per cent
C ₂ H ₄ O ₂ , $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, 2, 3 per cent	NaNO ₃ , 2, 4, 6 per cent

At various times isolations were made from each culture; but eggs were deposited by all of the individuals and there was no indication of the appearance of the male form.

Thus, neither a change in the kind nor the concentration of the culture medium, a constant or fluctuating change in the temperature, or a change in the chemical constitution of the medium, has been accompanied by the appearance of the male in *Proales decipiens*.

In June, 1919, five 'wild' specimens of this species were found in a culture jar filled recently with water from a small stream. These organisms were transferred gradually to malted milk, and

twenty-five eggs from the second generation in malted-milk solution isolated from each individual. All individuals thus separated deposited eggs of the usual thin-shelled type and there was no indication of the appearance of the male form.

To sum up, all individuals from the original clone have deposited eggs; no males have appeared during changes in chemical constitution of the medium, changes in temperature, or changes in kind and concentration of food. Progeny of individuals recently isolated from 'wild' ancestors likewise show a uniform production of females.

So, during the thirteen months that *Proales decipiens* has been under observation, reproduction has been entirely by parthenogenesis. Although no records have been kept of the generations produced by mass cultures during this entire period, an estimate of over 250 generations is conservative, calculated from records of sixteen to twenty-three generations produced from single ancestors during a period of thirty days' isolation. In this species continuous parthenogenetic reproduction for this number of generations has been accompanied by no evidence of weakening in the race. At the close of the experiment the range of egg production for 100 individuals in $\frac{1}{16}$ per cent malted milk at laboratory temperature was from 11 to 30 with an average of 19.50; the range of the length of life was 4 to 7 days, with an average of 5.80 days. One hundred individuals early in the history of the race under the same conditions, at about the same time in the year, showed a range in egg production of 4 to 23, with an average of 12.79 and a range in the length of life of 3 to 5 days, with an average of 4.65 days. The hatching of eggs in fresh culture medium takes place within twenty-four hours in 100 per cent of the cases, and, barring accidents, the young individual begins to deposit eggs within twenty-four hours after it is hatched. While no definite measurements can be made of the body size of the adult, owing to its constant movements, the dimensions reached by the adult in the later generations are as great as those shown by any of the individuals produced in the early history of the race. Continuous parthenogenesis for about 250 generations in *Proales*, then, has been accompanied by no

reduction in the viability of the eggs, by no retardation in the development, by no reduction in the range or average of egg production, by no reduction in the range or average of the life-cycle, and by no reduction in the size attained by the adult.

THE EFFECT OF SELECTION

As we have already seen, there is great variation in the length of life and the number of eggs produced in the different individuals of *Proales*, the life-cycle ranging from one to eight days, the egg production from one to thirty.

Are these variations the results of constitutional and hereditary differences, so that, if individuals showing the different rates of egg production and differences in length of life are isolated and allowed to reproduce we shall get stocks differing consistently in these respects?

This question of variation and the inheritance of variation in uniparental reproduction has been much studied, especially since Johannsen ('03), working with self-fertilizing beans, reached the conclusion that selection within the progeny of a single individual is ineffective and that complete regression occurs in the progeny of all individuals showing variation from the mean for the pure line. The same condition has been found not only in self-fertilizing plants, but in plants reproducing vegetatively by tubers, by grafting, and by buds and in animals reproducing asexually by budding (Lashley, in *Hydra*), by fission (Jennings, in *Paramecium*), and by parthenogenesis (Ewing, in *Aphids*).

By analyzing Johannsen's results statistically, Pearson ('10) found indications that inheritance might exist within the clone, since the correlation ratios diminished as the line of ancestry became more remote. If the genetic constitution of any individual of a pure line does not depend on its immediate ancestor, but on the type of the line to which they both belong, the coefficient of correlation between any individuals and any generation of their ancestors should be the same as the correlation between the individuals and their immediate ancestors. Later experimental work on Protozoa has given data agreeing with the conclusion that selection within a pure line is to a certain extent

effective in some cases. In *Stylonychia*, Middleton ('15) isolated from the progeny of a single individual two strains that differed in the rate of fission; in *Diffugia*, Jennings ('16) found strains that differed in a number of characters (number and length of spines, diameter of shell, etc.); in *Centropyxis*, Root ('17) isolated strains differing in number of spines and the size of shell; in *Arcella*, Hegner ('20) found another protozoan that showed the same phenomena, the lines differing in spine number and diameter of shell within the same clone.

In comparison with the mass of experimental data in the protozoa, a relatively small amount of work has been done on forms reproducing parthenogenetically, although in the species where no reduction in the number of chromosomes occurs, the reproduction is as typically uniparental as in the cases where an organism reproduces by fission.

Kelly ('13) tried by selection to alter the relation of the third to the fourth antennal joint in a parthenogenetic aphid (*Aphis rumicix*), but the work was carried on for only two generations and no effect produced.

Agar ('14) worked with three parthenogenetic Cladocera, *Simocephalus exspinosus*, *S. vetulus*, and *Daphnia obtusa*, and a parthenogenetic aphid, *Microsiphum antherinii*. In both species of *Simocephalus* an attempt was made to increase the body length by selection, but the coefficient of correlation between individuals and their ancestors showed no diminution as the scale of ancestors was ascended, and in *Daphnia* also there was "no evidence of inheritance of interclonal variation." In *Microsiphum antherinii*, however, there was a diminution in the intensity of the correlation from the parental to the grandparental relation, which indicated that selection within the clone had been effective. In a discussion of this part of the work, Agar suggests that such a conclusion must be stated tentatively, since in all the cases the progeny are produced viviparously and have short life histories, so that there is a comparatively short time to eradicate the effects of intra-uterine life. Ewing ('14 a, b, '16) studied various characters of *Aphis avenae* Fab., and in the first ten generations attempted by selection to increase

or decrease the ratio of the third and fourth antennal segments. He concluded that "Selection from among extreme variants does not alter the mean as obtained for the strain without selection. The offspring of an extreme variant may show, not a reversion to the mean of the line of a strain, but a reversion which swings pendulum-like much beyond the mean . . . only to be brought back to its former side of the mean-of-the-strain base line in the next generation." In the second paper this pendulum-like swinging of the mean for the offspring was tested through seven generations with the conclusion that "regression within a pure line of a parthenogenetic form does not follow Galton's law . . . but . . . is somewhat pendulum-like, swinging beyond the mean of the strain, or line." In the last paper extensive experiments carried on with the cornicles, the antennae, and the body length yielded results which pointed to the conclusion indicated in the earlier work, that selection in a parthenogenetic line had no lasting effect.

At the present time, then, experimental data on uniparental inheritance point in opposite directions, some work indicating that selection within a pure line is ineffective (Johannsen, Jennings, Ewing, and many botanists), other work, that selection is effective (Jennings, Middleton, Root, Hegner). There was some indication in Proales that an increase in length of life and in number of eggs produced had taken place during the time of cultivation in malted milk. A comparison of 100 organisms isolated in February, 1919, with 100 organisms isolated in June, 1919, shows an increase in average egg production from 12.79 to 23.43, with an increase in maximum production from 23 to 30. This increase in egg production was accompanied by an increase in individual range of life from 3 to 5 days to 2 to 7 days. Such increase in egg production and length of life suggested that by selection the maximum in both these might be increased still further. An experiment was therefore undertaken to test this. In this experiment in selection an attempt was made to eliminate variations in the conditions as much as possible; the food was prepared carefully from the same jar of malted milk, with water from the same spring throughout the

experiment, and all organisms after the fifth generation were kept in an electric constant temperature oven at 23° to 25°C. Since egg production and length of life are correlated as indicated above, the number of eggs produced rather than the length of life was decided upon as a more reliable measure of performance, and an attempt was made by selecting in each generation the individual producing the highest number of eggs to increase the maximum number of eggs produced by any individual beyond thirty, a number frequently obtained in individuals chosen at random.

The progenitor for this selection work was chosen at random from the progeny of a mother producing twenty-four eggs. The individual thus chosen produced twenty-seven eggs, designated as the first generation. All these eggs hatched, but two of the young organisms died before producing eggs. The minimum number of eggs produced by any of the twenty-five sister individuals in the first generation was 5, the maximum 28, the average 18.68.

A check-line was reared under the same experimental conditions and isolations made at the same time as in the selection experiment, but the progenitor for the next generation was chosen at random rather than by an inspection of the egg-depositing record. In the first generation the check-line deposited 18 eggs, a number slightly below the average, 18.68, for the selection line. The individual range for the length of life in the selection experiment was two to six days with an average of 4.36 days as compared with an length of life of four days in the check. During the first generation the selected line showed a much higher maximum and average number of eggs, and a greater average length of life than the non-selected line.

The further course of the experiment, through fifteen generations of selection, is shown in table 3. Throughout the experiment the individual producing the highest number of eggs was chosen to continue the selected lines, while for the control line an individual was taken at random.

As will be seen in table 3, fifteen generations of selection of the individual producing the highest number of eggs did not bring

about any clear increase either in the maximum number of eggs produced, the individual range of life, or the average length of life. In four generations the average length of life in the selected line is greater than the length of life in a line of individuals chosen at random. In three generations the average number of eggs produced by the selected line is greater than the number produced by an individual in a non-selected line. In all other cases

TABLE 3

A comparative table of the selected and non-selected lines of Proales, showing the numbers of eggs produced and the length of life

GENERATION	SELECTED INDIVIDUALS						NON-SELECTED INDIVIDUALS	
	Number of eggs produced			Length of life in days			Number of eggs produced	Length of life in days
	Minimum	Maximum	Average	Minimum	Maximum	Average		
1	5	28	18.68	2	6	4.36	18.00	4
2	2	27	13.85	2	6	3.42	16.00	4
3	2	30	17.00	2	6	4.04	17.00	4
4	6	28	19.00	2	6	4.02	21.00	5
5	4	26	17.14	1	4	3.71	18.00	4
6	1	27	18.56	1	5	3.60	19.00	4
7	3	28	15.57	2	6	4.23	22.00	6
8	3	28	18.03	2	6	4.97	20.00	5
9	1	29	13.84	1	7	4.80	25.00	6
10	2	28	20.14	3	7	5.22	20.00	4
11	8	26	17.92	3	6	4.68	24.00	6
12	7	26	17.03	3	5	4.42	19.00	5
13	4	27	17.20	2	7	4.64	16.00	4
14	6	26	17.66	3	6	5.08	22.00	6
15	3	25	16.70	3	6	4.66	25.00	7

the selected line shows an average either equal to or below the numbers produced in a non-selected line. These results indicate that at this time selection for a greater number of eggs and a longer average life-cycle in *Proales* is ineffective. The results obtained from this selection work do not offer any explanation for the great variation in maximum and average length of life, in range of individual life-cycle, and in maximum and average egg production mentioned earlier as occurring between the

earlier and the later periods of the work. Such an increase may have been due to the greater degree of adjustment to the environment in the individuals constituting the race after cultivation for six months in malted milk.

THE EFFECT OF ALCOHOL IN RELATION TO INHERITANCE

Can the inherited characteristics of organisms be changed by environmental agents? Particularly, is it possible by poisons, by extremes of temperature, or by excessive concentration of salts in themselves non-toxic, so to injure germ cells that later generations will be weak, abnormal, or essentially different from their parents?

This phase of the general problem of the causes of variation in organisms has been attacked more extensively in mammals and birds than in any other members of the animal kingdom. The very interesting and important sociological significance of a limited part of the problem, namely, the effect of alcohol on human welfare, has resulted in the bringing together of a large mass of data, unscientifically collected and arranged for the most part, pointing in a general direction too familiar to require setting forth here. The larger mass of the experimental data on the same subject has been collected during the last few years on guinea-pigs and the domestic fowl. Stockard ('18), working with guinea-pigs, has studied for over seven years the behavior of these animals when subjected to the influence of alcohol. Not only has the effect of alcohol on the individual animal been studied, but also the effect of alcohol on the progeny when matings were made between alcoholized animals, between alcoholized animals and normal animals, and between the progeny of alcoholized animals and normal animals as far as the third generation. In regard to the effect of the alcohol on the individual animal the earlier work is summed up as showing "that the germ cells in either the male or the female mammal may be changed or affected by a chemical treatment administered to the body of the animal" (L 120). The progeny of animals thus treated "showed more or less marked deviation from the normal in many definitely measurable qualities, such as their mortality records, structural

appearance, nervous reactions, and ability to reproduce" (p. 120). When the progeny of alcoholized animals, themselves not subjected to the treatment, were mated the data were such that "it may be concluded that animals as far as three generations removed from the direct alcohol treatment are still differentiated from the control in regard to the weight of the litters in which they are born, the tendency of the matings to result in failure" (p. 164). In general, then, the alcoholization of guinea-pigs produces some change in the germ cells which is still evident in the third generation of non-treated progeny, even when normal germplasm is thrown into the hereditary stream at each mating.

In the domestic fowl, Pearl ('17 a, b, c,) has used the same general method of treatment, in this case treating the birds for only one hour per day. In appearance "the treated animals themselves are not conspicuously worse or better than their untreated control sisters and brothers" (p. 185). The capacity to reproduce was not influenced since "neither the total amount nor the distribution of egg production were significantly different in the treated birds from what they were in the controls" (p. 186). But "the proportion of fertile eggs . . . was materially reduced in the matings in which one or both individuals had been treated" (p. 294). At the close of the account of the experiment the conclusion was reached that "There is no evidence from these experiments that the treatment of individual fowls with ethyl alcohol had any deleterious effect upon those germ cells which form zygotes. The treatment rendered many germ cells incapable of forming zygotes at all, but those which did form zygotes had plainly not been injured in any way" (p. 295).

Thus among the higher organisms the evidence points in opposite directions, that secured from mammals indicating the persistence of the influence of the environmental agent as far as the third generation of the untreated progeny, that secured from birds not indicating that there is a change produced in the germ cells by the subjection to alcohol. Among the lower organisms Whitney ('12) has studied the effect of alcohol on the rotifer *Hydatina senta*. He reared the organisms in culture fluid plus $\frac{1}{4}$, $\frac{1}{2}$, and 1 per cent ethyl alcohol for twenty-eight

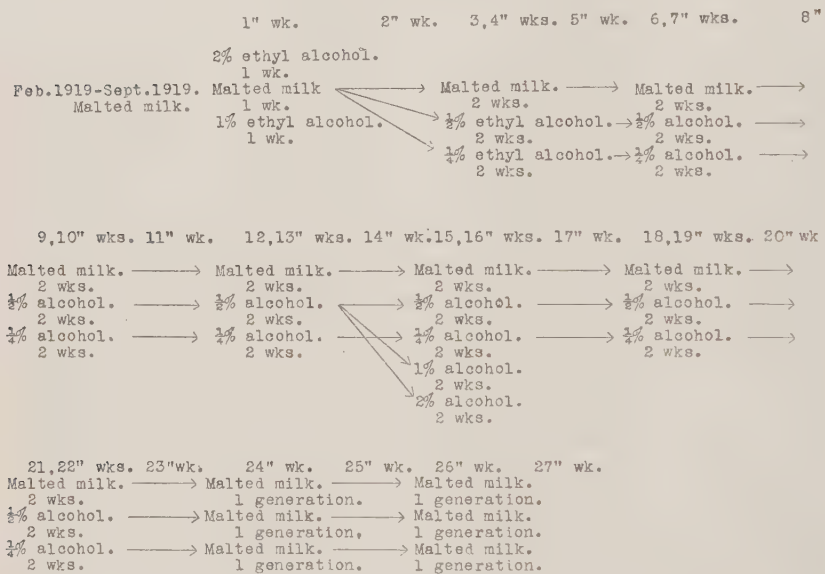
generations, starting each new generation with a daughter from an alcoholized mother. At the end of the experiment progeny of these alcoholized lines were returned to normal culture solutions to test the retention of the influence of the alcohol. In regard to the production of eggs, it was found that "The rate of reproduction was lower in the alcoholic strains than in the control and it was proportionally lowered according to the amount of alcohol used." But after the return of the progeny to normal culture solutions it appeared that "The weaknesses developed by the parental use of alcohol are partially eliminated in the first generation after the alcohol has been removed, and practically completely eliminated at the end of the second generation after the alcohol has been removed." In so far as such a comparison can be made, the rotifer *Hydatina senta* reacts to alcohol in much the same way as the domestic fowl.

The question of the effect of alcohol on germ cells is of such importance that it seemed worth while to test it again as thoroughly as possible in another of the lower organisms. *Proales* seemed particularly favorable for this work, since it will thrive in a medium of comparatively definite composition, passes through the life-cycle in a short time, and produces a large number of eggs with a high percentage of viability. In preliminary study it was found that individuals of *Proales* subjected to the fumes of ethyl alcohol in solutions of varying percentages showed an effect equal to that on individuals reared in culture solutions to which an equal per cent of alcohol had been added directly. The same thing has been found to be true for infusoria by Estabrook ('10), working on *Paramecium*, and in many other experiments carried out in this laboratory. Throughout the experiment with *Proales* the organisms have been isolated in normal $\frac{1}{16}$ per cent malted milk and placed on supports in closed Stender dishes. In the bottom of each culture dish sufficient absolute alcohol was added to 100 cc. tap-water to give the percentage indicated in the particular experiment cited. These alcohol solutions were changed each day, the adult organisms transferred to fresh culture fluid, and if individual data were to be secured on the eggs, they were transferred to fresh culture fluid also.

In September, 1919, 100 individuals were chosen at random from the mass cultures established in August, these being originally all descendants of a single individual. These were reared under normal conditions until they had each deposited three or more eggs. Three eggs from each of these 100 organisms were used to start the experiment; one from each individual was placed in a culture dish with 1 per cent alcohol in the solution used in the bottom, another in a dish with 2 per cent alcohol, and a third in a dish with tap-water to serve as a check for the alcohol lines. The cultures were examined daily, the alcohol solutions changed, and the adults transferred to fresh food. At the end of the first week 100 organisms were isolated from each main line and the length of life and the egg production determined in each. (See table 4 for a diagrammatic scheme of the alcohol experiment.) At the end of the first week the range in egg production for the 100 individuals from the 1 per cent alcohol line was 1 to 7, with an average of 2.85, while the range in the length of life was 2 to 6 days, with an average of 3.88 days; in the line reared subjected to 2 per cent alcohol the range in egg production was 1 to 6, with an average of 1.84; the range of individual life was 2 to 5 days, with an average length of life of 4.19 days; in the check line the egg production ranged from 6 to 30, with an average of 20.86, while the range in the length of life was 3 to 5 days, with an average of 4.78 days. At the end of the first week individuals from each of the alcohol lines showed a decided reduction in the power to deposit eggs; those subjected to 1 per cent alcohol depositing an average of 2.85 eggs, and those subjected to 2 per cent an average of 1.84 eggs, as compared with an average deposit of 20.86 eggs in individuals reared under normal conditions. The average length of life did not show as great a reduction; subjected to 1 per cent alcohol, the individuals lived an average of 3.88 days; to 2 per cent, an average of 4.19 days, while those under normal conditions had an average length of life of 4.78 days. Thus there is a marked reduction in the egg-laying capacity, unaccompanied by so great a reduction in the average length of life, early in the history of a line reared in alcohol. The viability of the eggs produced in these per-

centages of alcohol was so low (less than 25 per cent) that it soon became evident that it would be impossible to continue the lines indefinitely. Hence the line in 2 per cent alcohol was discontinued and the progeny produced in 1 per cent alcohol divided into two lots, one to be subjected to $\frac{1}{4}$ per cent alcohol, the other to $\frac{1}{2}$ per cent. The organisms were reared for two weeks over these percentages of alcohol; isolation of eggs for new generations were made whenever the age of the majority of adults

TABLE 4
Diagrammatic scheme of alcohol experiment



seemed to warrant. At the end of this time (five weeks) 100 individuals from each line were isolated and the egg production and length of life determined. In the $\frac{1}{4}$ per cent alcohol the range of egg production was from 4 to 22, with an average of 14.25; the range of life from 3 to 7 days, with an average length of life of 5.83 days in the $\frac{1}{2}$ per cent alcohol the range in egg production was from 1 to 10, with an average of 3.98; the range of life from 4 to 7 days, with an average of 6.09 days; in the controls the egg production ranged from 14 to 30, with an average of 14.10 eggs; the length of life from 3 to 7 days, with an average

of 6.17 days. When these percentages of alcohol are employed, the reduction in the number of eggs deposited is not so marked as in higher percentages, but the difference between the alcohol lines and the line reared under normal conditions is still great. There is little reduction in the average length of life in either percentage of alcohol, just as was the case where 1 per cent and 2 per cent were used.

The alcohol experiment was carried on for twenty-seven weeks in the way just indicated; that is, 100 individuals each subjected to $\frac{1}{4}$ and $\frac{1}{2}$ per cent alcohol solutions, and 100 controls, were allowed to reproduce for a period of two weeks, when isolation of 100 specimens was made from each line and the egg deposit and length of life for all the individuals determined under each of the three conditions; reproduction continued for another two-week period, then another isolation made, etc., until the end of the twenty-third week. At this time the alcohol cultures were discontinued and progeny from both alcohol lines were returned to malted-milk solution without alcohol. In this way it was determined whether the effects of $\frac{1}{4}$ and $\frac{1}{2}$ per cent alcohol, which had been acting continuously on the progenitors of both lines for twenty-one weeks, had any lasting effect when the progeny were returned to malted milk. At the beginning of the fifteenth week progeny of the line reared in $\frac{1}{2}$ per cent alcohol were isolated and reared in 1 per cent and $1\frac{1}{2}$ per cent alcohol for a period of two weeks and an isolation made at the end of this time to determine if under the continuous action of $\frac{1}{2}$ per cent alcohol the organism had developed any degree of resistance to higher percentages.

The effect of the alcohol upon the organisms themselves was marked only in the higher percentages. In many cases in these percentages the increase in size, which usually continues until near the close of the life-cycle, never took place and the individuals were thin and attenuated; movement in many cases was reduced even in the young individual, and the adults became very sluggish. In the experiments where a low percentage of alcohol ($\frac{1}{4}$, $\frac{1}{2}$ per cent) was employed, the treated organisms resembled the untreated checks except in the number of eggs deposited.

An isolation made at the end of the tenth week showed that in the $\frac{1}{4}$ per cent alcohol line the range of egg production was from 1 to 19, with an average of 11.07. This is slightly lower than the results in the fifth week, where the range was from 4 to 22, with an average of 11.25. In the eleventh week the range of life was from 3 to 8 days, with an average of 6.46. In the fifth week the range of life had been from 3 to 7 days, with an average of 5.83 days. In the eleventh week in $\frac{1}{4}$ per cent alcohol the average and maximum production of eggs had fallen below that in the fifth week, but the maximum length of life at this time was the greatest attained by any individual throughout the entire study. A greater length of life under treatment with alcohol was noted by Stockard in one of his guinea-pigs, which lived seven years—an unusual time in that organism. Individuals subjected to $\frac{1}{2}$ per cent alcohol showed at this time a range in the egg deposit of 1 to 10, with an average of 5; this was the same as the range in the fifth week, but at that time the average was only 3.98. The range of life for this period was from 3 to 7 days, with an average of 5.70 days, as compared with a range of 4 to 7 days and an average of 6.09 days at five weeks. In malted milk under normal conditions the range of egg production at the eleventh week was 8 to 29, with an average of 19.70, while the range in life was 3 to 7 days, with an average of 6.48 days. At this isolation, just as in the previous one, there is a decided reduction in the number of eggs produced in both the alcohol lines and very little reduction in the average life-period.

At the beginning of the fifteenth week two new isolations of 100 individuals each were made from the $\frac{1}{2}$ per cent alcohol line; one of these was reared in 1 per cent alcohol; the other in $1\frac{1}{2}$ per cent. At the beginning of the seventeenth week isolations were made for the determination of the egg production and the length of life from the five lines, malted milk, $\frac{1}{4}$ per cent, $\frac{1}{2}$ per cent, 1 per cent, $1\frac{1}{2}$ per cent alcohol. At this time the line in $\frac{1}{4}$ per cent alcohol showed a range in egg production of 4 to 22, with an average of 10.80; a range in the length of life of 3 to 7 days, with an average of 5.88 days. The line in $\frac{1}{2}$ per cent alcohol showed an egg production ranging from 1 to 10, with an

average of 4.55, and a range of length of life of 2 to 7 days, with an average of 6.52 days. The line previously reared in $\frac{1}{2}$ per cent alcohol and transferred to 1 per cent only two weeks previous to this isolation showed a range in egg production from 1 to 7, with an average of 2.98, a range in length of life from 3 to 7 days, with an average of 5.22 days. The line recently transferred to $1\frac{1}{2}$ per cent alcohol showed a range in egg production of 1 to 6, with an average of 2, and a range in length of life of 2 to 7 days, with an average of 4.91 days. The line reared continuously in malted milk under normal culture conditions showed a range for egg production of 1 to 28, with an average of 19.26, and a range in the length of life of 3 to 7 days, with an average of 5.81 days.

In general during the twenty-one weeks in which individuals of a line of *Proales* were subjected continuously to the fumes of $\frac{1}{4}$ and $\frac{1}{2}$ per cent ethyl alcohol the maximum and average number of eggs produced was reduced in proportion to the percentage of alcohol used, but the average length of life was very little influenced. Table 5 gives a summary of the range and average egg production and the range and average length of life of all the individuals studied throughout the experiment. In neither of the lines subjected to $\frac{1}{4}$ and $\frac{1}{2}$ per cent alcohol is there a continual decrease in the average of either character studied throughout the successive generation.

Tests for inheritance of the effect of alcohol. At the beginning of the twenty-fourth week both alcohol lines were discontinued and their progeny returned to malted milk only. At the end of the first week in normal conditions (twenty-fifth week of the experiment) isolations of the second generation individuals were made for determining the egg production and average length of life. In the line descended from the progeny of the $\frac{1}{4}$ per cent alcohol group the range of egg production was from 9 to 27, with an average of 17.77, as compared with a range of 3 to 23, with an average of 12.33 eggs in the last generation in $\frac{1}{4}$ per cent alcohol. The range of life at this time was 3 to 7 days, with an average of 5.58 days, differing little from the range and average in the last isolation in $\frac{1}{4}$ per cent alcohol. In the line descended from individuals reared in $\frac{1}{2}$ per cent alcohol the range in egg produc-

tion was from 5 to 25, with an average of 13.57, as compared with a range of 1 to 11 and an average of 3.48 in the last generation in alcohol. The average length of life, 5.70 days, had increased very little over the average of 5.62 days in the last isolation. Individuals reared continually in malted milk showed at this time a range in egg production from 4 to 29, with an average of 18.56, and a range in length of life from 2 to 7 days, with an average of 5.56 (table 4).

TABLE 5

A comparison of the egg production and length of life of all isolations made of lines subjected to $\frac{1}{4}$ per cent and $\frac{1}{2}$ per cent alcohol and controls

NUMBER OF WEEK AT WHICH ISOLATION WAS MADE	LINE IN $\frac{1}{4}$ PER CENT ALCOHOL				LINE IN $\frac{1}{2}$ PER CENT ALCOHOL				CONTROLS			
	Egg production		Length of life		Egg production		Length of life		Egg production		Length of life	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
5th	4-22	14.25	3-7	5.83	1-10	3.98	4-7	6.09	4-30	24.10	3-7	6.17
6th	3-20	12.63	2-7	5.60	1-10	4.44	2-6	5.08	4-29	20.95	2-7	5.16
11th	1-19	11.07	3-8	6.46	1-10	5.00	3-7	5.70	8-29	19.70	3-7	6.48
14th	4-22	12.67	3-7	5.10	1-11	4.99	3-6	5.16	5-28	15.84	3-7	5.99
17th	4-22	10.80	3-7	5.88	1-10	4.55	2-7	5.52	4-28	19.26	3-7	5.81
20th	4-23	14.73	3-7	5.95	1-11	4.39	2-7	5.45	5-28	20.59	3-7	5.80
23rd	3-23	12.33	2-7	5.95	1-11	3.48	2-7	5.62	5-28	18.88	2-7	4.75
*												
25th	9-27	17.77	3-7	5.58	5-25	13.57	2-7	5.70	4-29	18.56	2-7	5.56
27th	12-30	21.80	3-7	5.98	8-29	19.92	3-7	5.37	11-30	19.50	4-7	6.06

* Indicates time when alcohol cultures were transferred to malted milk.

Thus, individuals of the second generation after the return to normal conditions showed a marked increase in egg production; in other words, only a partial retention of the influence of the alcohol; and the averages for the generation whose ancestors were subjected to alcohol approach those individuals continually reared in malted milk.

Isolations of the third generation made under the same conditions as have just been described at the beginning of the twenty-seventh week, show an average egg production of 21.80 for descendants of individuals subjected to $\frac{1}{4}$ per cent alcohol, and 19.92 for those subjected to $\frac{1}{2}$ per cent, as compared with an average

of 19.50 for checks reared continuously in malted milk. At this time, after three generations spent in malted milk, all the effects of alcohol upon egg production has been lost.

To summarize: In *Proales decipiens* individuals of a line subjected to the fumes of $\frac{1}{4}$ and $\frac{1}{2}$ per cent ethyl alcohol continuously for nineteen weeks show a decided reduction in egg production while under the influence of the alcohol, but their progeny, returned to normal conditions, regain the normal egg-producing power after the third generation.

SUMMARY

This paper is an account of the normal life-cycle of *Proales decipiens*, with experimental studies of the production of males, of the effects of selection during parthenogenetic reproduction, and of the effects of alcohol on inherited characteristics.

1. Statistics are given as to the length of life, the rate of reproduction, the number and kind of eggs deposited, with study of the variations in these matters. The animal lives about a week, then dies with characteristic symptoms of senility. During its life it produces several eggs per day, the number increasing to a maximum, then decreasing with the onset of old age.

2. Reproduction by parthenogenesis for about 250 generations gave no indication of reduction of vigor in the race in any respect.

3. During this period, no males appeared. Alteration of the environment by changes in the nature and concentration of the food, by changes in the temperature at which cultures were reared, and changes in the chemical constitution of the medium were not accompanied by the appearance of the male form. So far as known, the species may be quite without males.

4. An attempt to increase the egg deposit and average length of life through artificial selection carried on for three months, in fifteen generations, was without avail, placing this organism in the list with other parthenogenetic forms in which selection is ineffective.

5. Treatment with ethyl alcohol in a concentration of $\frac{1}{4}$ and $\frac{1}{2}$ per cent for twenty weeks reduced the number of eggs produced from an average of 15 to 24 in normal malted milk to an average

of 10 to 14 in $\frac{1}{4}$ per cent alcohol, and 3 to 5 in $\frac{1}{2}$ per cent alcohol, although the length of life was little influenced.

6. The reduction in egg deposit brought about by alcohol was not retained beyond the third generation of descendants restored to normal conditions of culture.

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